PATENT COOPERATION TREATY

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From the
INTERNATIONAL SEARCHING AUTHORITY

To:
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25/8

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	13 June 2005 (13-06-2005)
Applicant's or agent's file reference 08899871WO	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA2005/000211	18 February 2005 (18-02-2005)	18 February 2004 (18-02-2004)

International Patent Classification (IPC) or both national classification and IPC
IPC7: C12Q-1/68, C12P-19/34

Applicant
CENTRE FOR ADDICTION AND MENTAL HEALTH ET AL

1. This opinion contains indications relating to the following items :

- [X] Box No. I — Basis of the opinion
- [X] Box No. II — Priority
- [ ] Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV — Lack of unity of invention
- [X] Box No. V — Reasoned statement under Rule 43*bis*.1(a)(I) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI — Certain documents cited
- [ ] Box No. VII — Certain defects in the international application
- [X] Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**
If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/CA	Date of completion of this opinion	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476	12 May 2005	Qianfa Chen (819) 994-1374

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/CA2005/000211

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   [X] the international application in the language in which it was filed

   [ ] a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of :

   a. type of material

      [X] a sequence listing

      [ ] table(s) related to the sequence listing

   b. format of material

      [X] on paper

      [X] in electronic form

   c. time of filing/furnishing

      [ ] contained in the international application as filed.

      [ ] filed together with the international application in electronic form

      [X] furnished subsequently to this Authority for the purposes of search.

3 [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statement that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments :

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/CA2005/000211

Box No. II Priority

1. [ ] The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43bis.1 and 64.1) is the claimed priority date.

2. [ ] This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary :

It has not yet been possible to consider the validity of the priority claim because the Authority does not have in its possession a copy of the earlier application whose priority has been claimed. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(I) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-9, 11, 12, 15 and 20-22	YES
	Claims	10, 13, 14 and 16-19	NO
Inventive step (IS)	Claims		YES
	Claims	1-22	NO
Industrial applicability (IA)	Claims	1-22	YES
	Claims		NO

2. Citations and explanations :

Reference is made to the following documents:

D1: US 6,605,432 B1 (HUANG, T.), 12 August 2003.

D2: YAN, P. et al. J Nutrition. August 2002, Vol.132, No.8 (Suppl), Pages 2430S-2434S.

D3: WO 03/027259 A2 (WANG, Z.), 3 April 2003.

D4: WO 00/26401 A1 (ISSA, J-P. et al.), 11 May 2000.

D5: WO 03/064701 A2 (SLEDZIEWSKI, A. and SCHWEIKHARDT, R.G.), 7 August 2003.

D6: CHOTAI, K. and PAYNE, S.J. J Med Genet. June 1998. Vol.35, No.6, Pages 472-475.

D7: US 2003/0099997 A1 (BESTOR, T.H.), 29 May 2003.

D1 describes a method for the identification of DNA methylation patterns in cancer cells. A nucleic acid sequence of interest is digested with a restriction endonuclease enzyme *MseI* which digests nucleic acid sequences into fragments in which CpG islands are preserved. These fragments are ligated to linkers and subsequently digested with a methylation sensitive enzyme (e.g., *BstUI*) resulting in a digestion product comprising methylated CpG island loci. The digestion product is amplified and labelled to form amplicons which are used to screen a plurality of nucleic acid fragments affixed to a solid support. The presence or absence of labelled amplicons bound to the plurality of nucleic acid fragments of the screening array, from both cancer and normal cells, is then determined thereby identifying the methylation patterns of the cancer cells.

D2 describes a high-throughput microarray technique for identifying changes in DNA methylation patterns in breast cancer and normal samples. DNA samples are treated with *MseI*, known to digest bulk DNA into small fragments but keep large GC-rich CpG island fragments relatively intact. These GC-rich CpG island fragments are ligated to linkers and restricted with methylation sensitive restriction endonucleases (e.g., *BstUI, HpaII, HhaI*) and then amplified by PCR using linker-primers. Methylated DNA fragments of the test samples are protected from digestion and amplified by linker-PCR, whereas the same unmethylated fragments in the control sample are digested and could not be amplified, and vice versa. The test and control amplicons are cohybridized to CpG

(Continuation on the Supplemental Box)

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made :

D. Description defects

The description does not comply with Article 5 of the PCT. The http internet addresses provided on page 38, line 24 is not a static electronic file. The information disclosed in said file can change and therefore is not reliable. As such, a person skilled in the art cannot practice the invention of the present application. A skilled person is not fully enabled to practice the invention of the present application.

A statement in an application, such as found on page 50, line 12, which incorporates by reference any other document, does not comply with Article 5 PCT.

E. Claim Defects

Claims 2 and 11 do not comply with Article 6 of the Patent Cooperation Treaty (PCT). Claims 2 and 11 refer to "a step of correcting for..." in the preamble while claims 2 and 11 define more than one method step.

Claim 2 does not comply with Article 6 of the Patent Cooperation Treaty (PCT). The term "step j)" has no antecedent.

Claim 3 does not comply with Article 6 of the Patent Cooperation Treaty (PCT). The term "step I)" has no strict antecedent in claim 2 on which claim 3 depends.

Claim 4 does not comply with Article 6 of the Patent Cooperation Treaty (PCT). The term "the CpG specific endonuclease" has no strict antecedent in claim 1 or 2 on which claim 4 depends.

Claims 5 and 13 do not comply with Article 6 of the Patent Cooperation Treaty (PCT) because the term "cocktail" implies more than one constitutive element while the instant claim defines a cocktail as comprising a single element.

Claims 7, 17 and 21 do not comply with Article 6 of the Patent Cooperation Treaty (PCT). The use of the expression "such as" attempts to give both broad and narrow meaning to the scope of the above claims.

Claims 8 and 18 do not comply with Article 6 of the Patent Cooperation Treaty (PCT) The term "said probe" has no strict antecedent in claims 1-6 and 10-16 on which claims 8 and 18 depend respectively. The term "said probe" should probably read "said label".

Claims 9 and 19 do not comply with Article 6 of the Patent Cooperation Treaty (PCT) The term "said fluorophore" has no antecedent in claims 1-6 and 10-16 on which claims 8 and 18 depend respectively.

Claim 21 does not comply with Article 6 of the Patent Cooperation Treaty (PCT). The use of the expression "for example" attempts to give both broad and narrow meaning to the scope of the above claim.

Applicant is requested to correct the following typographical errors:
in claim 1, step b), "the separately digesting" should probably read "separately digesting"; and
in claim 20, "capable hybridising" should probably read "capable of hybridizing".

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box V (page 1 of 3)

island microarray, and hypermethylation of multiple CpG islands in the test DNA (DNA from the breast cancer samples) is simultaneously detected using a two-colour (e.g., Cy3 and Cy5) fluorescence system.

D3 describes a method, for detecting whether the extent of methylation of one or more regions of DNA in a test sample is different from that of a control, comprising generating DNA fragments from at least one test sample of DNA by cleaving the methylation sites in the DNA that are not methylated while sparing methylation sites in the DNA that are methylated using methylation sensitive restriction endonuclease, ligating oligonucleotide linkers to the ends of the cleaved DNA fragments (e.g., *HpaII*-digested DNA fragments), amplifying the DNA fragments, differentially labelling the amplified DNA fragments (e.g., with Cy3 and Cy5 labels), hybridizing the amplified and labelled DNA fragments to one or more polynucleotides immobilized on a solid support, and comparing the amount of amplified DNA from the test sample that hybridizes to the immobilized polynucleotides versus that of a control, thereby detecting whether the extent of methylation of the one or more regions of DNA is different between the test sample and the control.

D4 describes a method for identifying a methylated CpG-containing nucleic acid including contacting a nucleic acid with a methylation sensitive restriction endonuclease (e.g., *SmaI, BssHII, HpaII, SacII, EagI* and *BstUI*) that cleaves unmethylated CpG sites, contacting the sample with an isoschizomer of the methylation sensitise restriction endonuclease, wherein the isoschizomer cleaves both methylated and unmethylated CpG sites. Oligonucleotide adaptors are ligated to the nucleic acid cleaved by the restriction nuclease, followed by the amplification of the ligated nucleic acid. The amplified digested nucleic acid is adhered to a membrane and hybridized with a probe of interest. The presence of the amplified products is indicative of the presence of the methylated CpG-containing nucleic acid.

D5 describes a method for profiling genome-wide methylation patterns by characterizing the methylation patterns of each sample set by means of one or a combination of techniques: methylated CpG island amplification (MCA); arbitrarily primed PCR (AP-PCR); restriction landmark genomic scanning (RLGS); differential methylation hybridization (DMH); and *Not I* restriction based differential hybridization. Specifically, MCA is a technique which is based on the sequential restriction enzyme digestion with a methylation sensitive restriction endonuclease and a methylation insensitive isoschizomers (e.g., *SmaI/XmaI*), linker/adaptor ligation and whole-methylated-genome PCR using the linker primers.

D6 describes a method for the diagnosis of Prader-Willi and Angelman syndromes. Both disorders are characterized at the molecular level by abnormal methylation of imprinted genes, at a common regions in chromosome 15q11-q13, including the small nuclear ribonucleoprotein N gene (SNRPN). The methylation status of the SNRPN sequences is detected by a differential digestion of the SNRPN sequences with a methylation sensitive restriction endonuclease *NotI* or with a methylation specific restriction endonuclease *McrBC*, followed by PCR amplification of the SNRPN sequences. The presence or absence of the abnormal methylation, as determined by the presence or absence of the amplified products of the SNRPN sequences, is used as a diagnostic marker for the diagnosis of Prader-Willi and Angelman syndromes.

D7 describes a method for detecting the presence of differential methylation between DNA from a first source and the corresponding DNA from a second source. The method is based on the digestion of the methylated DNA with methylation specific restriction endonuclease *McrBC*, and the digestion of the unmethylated DNA with methylation sensitive restriction endonuclease (e.g., *HpaII, HhaI, MaeII, BstUI* and *AciI*), followed by the detection of the formation of any hybrid DNA duplex between the DNA fragments digested with *McrBC* and DNA fragments digested with the methylation sensitive restriction endonuclease.

(Continuation on the Supplemental Box)

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box V (page 2 of 3)

A. Novelty

Claims 10, 13, 14 and 16-19 lack novelty and do not comply with Article 33(2) of the *Patent Cooperation Treaty (PCT),* as being anticipated by D1, D2, D3, D4 or D5 which separately describes a method for the detection of methylation of genomic DNA comprising the steps of restriction endonuclease digestion, adaptor ligation, amplification, labelling, array hybridization, and ratio determination. Therefore, D1, D2, D3, D4 or D5 is novelty destroying to claims 10, 13, 14 and 16-19.

Claims 1-9 and 22 meet the criteria set out in Article 33(2) of the Patent Cooperation Treaty (PCT), because none of the prior art describes the sequential digestion of genomic DNA with a) a methylation sensitive restriction endonuclease and followed with b) a methylation specific restriction endonuclease (claims 1-9), or the sequential digestion of genomic DNA with a) a first methylation sensitive restriction endonuclease, followed with b) a methylation insensitive restriction endonuclease, and further with c) a second methylation sensitive restriction endonuclease (claim 22) for the detection of methylation state of one or more nucleotide sequences.

Claims 11, 12, 15, 20 and 21 meet the criteria set out in Article 33(2) of the Patent Cooperation Treaty (PCT), because the closest prior art (D1, D2, D3, D4, D5, D6 or D7) does not teach the technical features defined in claims 1,1, 12, 15, 20 and 21.

B. Inventive steps

Claims 1-9 and 22 lack an inventive step and do not comply with Article 33(3) of the *Patent Cooperation Treaty (PCT)* as being obvious over D1, D2, D3, D4 or D5 in view of D6 or D7 and in further view of common general knowledge. D1, D2, D3, D4 or D5 has independently described a method for the detection of methylation of genomic DNA comprising the steps of digesting the DNA (both test DNA and control DNA) with a methylation sensitive restriction endonuclease, adding and ligating oligonucleotide adaptors to the ends of the digested DNA, amplifying the ligated DNA, labelling the amplified DNA (e.g., with Cy3 and Cy5 labels), hybridizing the labelled products with an array of probes, and determining the methylation of the DNA by visualizing the hybridization signals and comparing the signals from the test amplicons to the signals from the control amplicons. D1, D2, D3, D4 or D5 does not teach the use of a methylation specific restriction endonuclease. However, D6 or D7 describes a diagnostic method based on the detection of differential DNA methylation comprising treating the genomic DNAs (both test DNA and control DNA) with a methylation specific restriction endonuclease (e.g., *McrBC*). Further, treating a DNA with a first restriction endonuclease, and subsequently with a second restriction endonuclease, is a routine procedure in a laboratory in order to cleave the DNA at the desired sites and in order to produce the desired DNA fragments. Therefore, it would be obvious to a person skilled in the art to treat the ligated DNA either with a methylation specific restriction endonuclease (claims 1-9) as taught by D6 or D7, or with a methylation sensitive restriction endonuclease (claim 22) as described in D1, D2, D3, D4 or D5 respectively, subsequently after the first digestion with the methylation sensitive restriction endonuclease in D1, D2, D3, D4 or D5 respectively, in order to detect both methylated and unmethylated regions in a DNA sample thereby arriving at the method of claims 1-9, or in order to detect the extent of methylation in one or more regions of a DNA sample thereby arriving at the method of claim 22.

Claims 11, 12 and 21 lack an inventive step and do not comply with Article 33(3) of the *Patent Cooperation Treaty (PCT)*. Claim 10 on which claims 11 and 12 depend respectively are anticipated in view of the disclosure of D1 or D2. Dependent claims 11 and 12 and the independent claim 21 refer to a method for the correction for the effect of DNA sequence variation in a DNA methylation analysis using, as reference control, unmethylated

(Continuation on the Supplemental Box)

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box V (page 3 of 3)

genomic test DNA or unmethylated genomic control DNA prepared by amplification with a DNA polymerase, such as *Phi29* DNA polymerase. The correction for the effect of DNA sequence variation for any comparative analysis of DNA by applying a reference in the analysis is a standard technique that is routinely used in any such analysis. Specifically, any DNA prepared in vitro by a DNA polymerase is inherently unmethylated. Consequently, such preparation of unmethylated genomic test or control DNA does not involve any inventive ingenuity. Therefore, claims 11, 12, and 21 lack an inventive step having regard to D1 or D2 in combination with common general knowledge.

Claim 15 lacks an inventive step and does not comply with Article 33(3) of the *Patent Cooperation Treaty (PCT)*. Claim 14, on which 15 depends is anticipated in view of the disclosure of D1 or D2 which separately describes the use of a DNA restriction endonuclease (e.g., *MseI*) that is known to digest bulk DNA into small fragments but keep large GC-rich CpG island fragments relatively intact (i.e., selective for A/T rich sequences over C/G sequences). The substitution of the restriction *MseI* of D1 or D2 with any commercially available restriction enzyme which is selective for A/T rich sequences over C/G sequences is held to be an obvious substitution and does not involve any inventive ingenuity. Therefore, an inventive step cannot be acknowledged for claim 15 having regard to D1 or D2 in combination with common general knowledge.

Claim 20 lacks an inventive step and does not comply with Article 33(3) of the *Patent Cooperation Treaty (PCT)* having regard to D1, D2, D3, D4 or D5 in combination with D6 or D7 and in further view of common general knowledge. Claim 20 is directed to a kit comprising a list of elements, each of which is commercially available and is well known in the art. For example, D1, D2, D3, D4 or D5 respectively describes test or control genomic DNA nucleotide sequences, linker/adaptors, hybridization probes, and the reagents and enzymes for amplifying nucleotide sequences. D1, D2, D3, D4, D5, D6 or D7 separately describes methylation sensitive restriction endonucleases (e.g., *BstUI and HpaII*). D1 or D2 separately describes restriction endonuclease *MseI* (a frequent cutting restriction endonuclease). D3 describes microarrays capable of hybridizing to nucleotide sequences and software for displaying and analysing the sequences hybridized to the microarrays. D6 or D7 separately describes methylation specific restriction endonuclease *McrBC*. It would be obvious to a person skilled in the art to prepare a kit by a simple aggregation of old and known compounds/elements. In the absence of a novel or inventive use of the kit, an inventive step cannot be acknowledged for a kit which comprises compounds/elements each of which is old and known.

C. Industrial Applicability

Claims 1-22 have industrial applicability as defined under Article 33(4) of the *Patent Cooperation Treaty (PCT)*.